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CHEMICAL STUDIES OF THE MECHANISM OF THE NARCOSIS INDUCED BY HYPNOTICS. II. THE SYNTHESIS OF COLORED DERIVATIVES OF PHENOBARBITAL

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE PHYSICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

1934

BY
ALAN EUGENE PIERCE

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Chemical Studies of the Mechanism of the Narcosis Induced by Hypnotics. II. The Synthesis of Colored Derivatives of Phenobarbital

By Alan E. Pierce

The pharmacological and histological study of of a hypnotic which shows the property of selectively staining nerve cells may be found to give insight into the mechanism of hypnosis. With the synthesis of such a dye-hypnotic as an object, the work described in this paper consisted of the preparation of several colored derivatives of phenobarbital, or 5-phenyl-5-ethylbarbituric acid, $(C_6H_5)(C_2H_5)\underline{C}CONHCONHCO$, which is itself a white compound. Several investigators[1] have attempted the preparation of physiologically active, colored derivatives of cocaine or procaine with varying degrees of success. It was hoped that phenobarbital would also be suitable for such a study.

In the first paper of this series,[2] the syntheses of four dye derivatives of phenobarbital by means of coupling diazotized 5-*m*-aminophenyl-5-ethylbarbituric acid with various phenolic compounds separately were described. Not one of these products, however, exhibited satisfactory hypnotic properties.

In the present investigation two colored derivatives of phenobarbital were prepared by the coupling of diazotized 5-*m*-aminophenyl-5-ethylbarbituric acid with *m*-phenylenediamine to form 5-*m*-(2,4-diaminophenylazo)-phenyl-5-ethylbarbituric acid, $(NH_2)_2C_6H_3N{=}NC_6H_4(C_2H_5)\underline{C}CONHCONHCO$, and with 5-*m*-hydroxyphenyl-5-ethylbarbituric acid to form *x*-hydroxyazobenzene-*x*,3′-bis-(5-ethylbarbituric acid), $\underline{CONHCONHCOC}(C_2H_5)C_6H_4$-$N{=}N{-}(OH)C_6H_3(C_2H_5)\underline{C}CONHCONHCO$. A third colored derivative is *m*-diazoamino-5-phenyl-5-ethylbarbituric acid, $\underline{CONHCONHCOC}(C_2H_5)C_6H_4$-$N{=}N{-}NHC_6H_4(C_2H_5)\underline{C}CONHCONHCO$. In this synthesis it was hoped by means of linking two ureide nuclei to ensure the preservation of the physiological effect of phenobarbital.

In addition to 5-*m*-nitrophenyl-5-ethylbarbituric acid, obtained by the nitration of phenobarbital, the para nitro compound was isolated in small quantity and identified, but was not used in the syntheses.

For the pharmacological study of the compounds prepared in this work the writer is indebted to several investigators. The work was done in part by Dr. A. L. Tatum, Professor of Pharmacology, University of Wisconsin, and in part by Dr. H. A. Shonle and Mr. E. E. Swanson of the Eli Lilly Company, Indianapolis. Intraperitoneal administration of the sodium salts of the various compounds to rabbits and white mice produced no sedative action without undesirable effects. Neither the three colored compounds nor 5-*m*-hydroxyphenyl-5-ethylbarbituric acid were effective in sub-lethal doses. The 5-*p*-nitrophenyl-5-ethylbarbituric acid was not tested, since the meta isomer has been found to be physiologically inert.[3]

Experimental

5-*m*-Nitrophenyl-5-ethylbarbituric Acid.—The method of Bousquet and Adams[3] was followed in the nitration of

(1) (a) Ehrlich and Einhorn, *Ber.*, **27**, 1872 (1894); (b) Fulton, *Am. J. Physiol.*, **57**, 158 (1921); (c) Gardner and Joseph, *J. Am. Chem. Soc.*, **57**, 901 (1935).

(2) Rising, Shroyer and Stieglitz, *ibid.*, **55**, 2818 (1933).

(3) Bousquet and Adams, *ibid.*, **52**, 224 (1930).

phenobarbital. Several recrystallizations of the crude nitrated material from 95% alcohol gave a 60% yield of 5-*m*-nitrophenyl-5-ethylbarbituric acid, m. p. 283–284°. The position of the nitro group in this product has been previously proved.[4]

5-*p*-Nitrophenyl-5-ethylbarbituric Acid.—The alcoholic mother liquors from the recrystallizations of 5-*m*-nitrophenyl-5-ethylbarbituric acid were evaporated to dryness. The residue (7.0 g. from 20 g. crude nitro-ureide) was dissolved in the least possible amount of dilute sodium hydroxide solution, and, with rapid stirring of this solution, enough dilute hydrochloric acid to neutralize three-fourths the alkali used was slowly added to the solution. The white precipitate which formed was brought upon a filter, washed with water, and recrystallized several times, from either 95% alcohol or water, until a product of m. p. 216–217° was obtained. Further crystallization from alcohol, water or toluene did not change this melting point; yield 1.2 g. *Anal.* Calcd. for $C_{12}H_{11}N_3O_5$: C, 51.96; H, 4.00; N, 15.17. Found: C, 52.12, 51.93; H, 4.08, 3.99; N, 15.17, 15.22.

The position of the nitro group in the substance just described was determined through alkaline permanganate oxidation of the material. The pure product (5.0 g.) was dissolved in 300 cc. of 1% potassium hydroxide solution. This solution was refluxed for twelve hours, as 25.0 g. of potassium permanganate was added in portions. When all permanganate color of the reaction mixture had disappeared, the solution was filtered from the manganese dioxide, evaporated to 40 cc., strongly acidified with concentrated hydrochloric acid and chilled. The product which separated was brought upon a filter, washed with water, and recrystallized from hot water with Norite added, yielding 1.2 g. of white crystals, m. p. 239–239.5°. The yield was 40% of the theoretical amount of nitrobenzoic acid. A mixture of the oxidation product and pure *p*-nitrobenzoic acid melted at 239–240°. The oxidation product was reduced with ammonium sulfide, yielding a white crystalline substance, m. p. 186–187°. A mixture of the reduction product and pure *p*-aminobenzoic acid melted at 186–187°.

5-*m*-Aminophenyl-5-ethylbarbituric Acid.—This was prepared by the catalytic reduction of 5-*m*-nitrophenyl-5-ethylbarbituric acid according to the directions of Bousquet and Adams[3]; yield 90%, m. p. 208–209°.

5-*m*-Hydroxyphenyl-5-ethylbarbituric Acid.—Bousquet and Adams[3] attempted to prepare this substance. Our procedure follows. 5-*m*-Aminophenyl-5-ethylbarbituric acid (2.47 g.) was diazotized at 5° in a solution of 5 cc. of concentrated hydrochloric acid in 50 cc. of water, a 10% sodium nitrite solution being used. At the end of the diazotization the excess nitrous acid present was destroyed by the addition of urea. The solution was now heated rapidly and boiled as long as nitrogen was evolved. During this heating the solution assumed a red color. The beaker containing the reaction mixture was vigorously shaken in an ice-bath until a red gummy substance gathered on the sides of the vessel and the liquid itself was practically colorless. At this point the mixture was filtered quickly, and pink crystals soon formed in the filtrate. This product was brought on a filter, washed with water, and recrystallized from hot water with Norite added. The white crystalline material so obtained was a dihydrate, but was easily dehydrated at 110°; yield 77%, m. p. 199.5–200° (anhydrous product). *Anal.* (anhydrous product). Calcd. for $C_{12}H_{12}N_2O_4$: C, 58.04; H, 4.88; N, 11.29. Found: C, 57.96, 58.06; H, 4.93, 4.88; N, 11.27, 11.48. Hydrate, calcd. for $2H_2O$, 12.68. Loss of weight at 110°, 12.68.

5 - *m* - (2,4 - Diaminophenylazo) - phenyl - 5 - ethylbarbituric Acid.—5-*m*-Aminophenyl-5-ethylbarbituric acid (2.47 g.) was diazotized in the manner described in the preparation of 5-*m*-hydroxyphenyl-5-ethylbarbituric acid, only 2.5 cc. of concentrated hydrochloric acid being used. To the solution of the diazonium salt was added a cold solution of 1.9 g. of *m*-phenylenediamine dihydrochloride in 20 cc. of water. After this solution was stirred for fifteen minutes a solution of 6.9 g. of sodium acetate in 50 cc. of water was added slowly, the reaction mixture being kept well cooled. After it was stirred for another half hour, the mixture was heated to boiling and filtered. The orange crystals remaining on the filter were purified by solution in hot dilute hydrochloric acid, followed by the addition of sodium acetate to this solution. The tarry material which first formed was separated by quick filtration of the solution. The addition of more sodium acetate to the filtrate caused the separation of the desired product. After a second solution and precipitation in this manner the final product was crystallized from hot water, in which it is slightly soluble. The pure compound consisted of orange crystals, m. p. 221–222°, yield 70%. *Anal.* Calcd. for $C_{18}H_{18}N_6O_3$: C, 58.98; H, 4.96; N, 22.95. Found: C, 58.77, 58.88; H, 5.00, 4.99; N, 22.89, 23.00.

Coupling of Diazotized 5-*m*-Aminophenyl-5-ethylbarbituric Acid with 5-*m*-Hydroxyphenyl-5-ethylbarbituric Acid.—5-*m*-Aminophenyl-5-ethylbarbituric acid (1.0 g.) was suspended in 50 cc. of water and diazotized as previously described, 2.0 cc. of concentrated hydrochloric acid being used. To the solution of the diazonium salt, made just alkaline to litmus with dilute sodium hydroxide solution, was added dropwise with stirring a cold solution of 1.1 g. of 5-*m*-hydroxyphenyl-5-ethylbarbituric acid dihydrate in 25 cc. of water to which just enough alkali had been added to dissolve the ureide. After fifteen minutes, a dye was precipitated from solution by the gradual acidification with normal hydrochloric acid, stirring being continued. The yellow solid was brought upon a filter, washed with water, and purified by solution in dilute sodium hydroxide, and reprecipitation with hydrochloric acid; yield 92%, dec. approx. 280°.

Anal. Calcd. for $C_{24}H_{22}N_6O_7$: C, 56.89; H, 4.38; N, 16.60. Found: C, 56.70, 56.89; H, 4.54, 4.42; N, 16.10, 16.05.

***m*-Diazoamino-5-phenyl-5-ethylbarbituric Acid.**—5-*m*-Aminophenyl-5-ethylbarbituric acid (2.0 g.) was suspended in 100 cc. of water and diazotized as previously described, using 2.0 cc. of concentrated hydrochloric acid. To the solution of the diazotized amine was added a cold solution of 1.9 g. of 5-*m*-aminophenyl-5-ethylbarbituric acid in 75 cc. of water containing 1 cc. of concentrated hydrochloric acid. A solution of 4.9 g. of sodium acetate in 25 cc. of

(4) (a) Bush and Johnson, *J. Am. Chem. Soc.*, **55**, 3894 (1933). (b) Rising and Pierce, *ibid.*, **55**, 3895 (1933); the melting point of the nitroureide was erroneously stated in this communication to be 279–280°.

water was added dropwise with stirring. The yellow precipitate which separated was brought upon a filter, washed with water, and purified by solution in dilute alkali and reprecipitation by the slow addition of dilute hydrochloric acid to a point faintly acid to litmus. The solid was collected, washed and dried at 110°; yield 75%, dec. 210°.

Anal. Calcd. for $C_{24}H_{23}N_7O_6$: C, 57.00; H, 4.59; N (total), 19.41; N (diazoamino), 5.55. Found: C, 56.80, 56.98; H, 4.57, 4.60; N (total), 19.32, 19.33; N (diazoamino, according to procedure to be described), 5.47, 5.44.

The Microdetermination of Diazoamino Nitrogen

The method of Houben-Weyl[5] for analyzing diazoamino compounds was modified and adapted to the micro scale. The modified method should be applicable to substances which evolve nitrogen when heated with hydrochloric acid.

The apparatus (Fig. 1) was constructed from a piece of 10-mm. Pyrex glass tubing 15 cm. long. When in use, it was connected to the carbon dioxide generator, at the end near A, and to the azotometer, at the end near B, by flexible connections, each made by joining together several short pieces of 1-mm. capillary tubing with clean rubber connections. A precision microazotometer, described by Pregl,[6] was used.

The sample to be analyzed was deposited in the bulb A and 0.5 cc. of freshly boiled concentrated hydrochloric acid in the bend of the tube at B. The tube was supported in the position shown, connected with generator and azotometer, and thoroughly flushed with a stream of pure carbon dioxide, until the bubble residues in the azotometer were no longer of significant size.[6] The gas stream was adjusted to the rate of one bubble per second, and the apparatus tilted so that the acid in B ran down upon the sample in A. By heating the chamber A, the acid was boiled for a few minutes, decomposing the diazoamino compound with the liberation of nitrogen. The source of heat was withdrawn and the tube swept out with carbon dioxide. The collection and measurement of nitrogen and the subsequent calculation were made according to Pregl's directions.

This micro method was tested by analyzing four diazoamino compounds, whose purity was initially established by duplicate total nitrogen determinations. In the analyses for diazoamino nitrogen the sample size varied between 2.5 and 6 mg. The results are shown in Table I.

TABLE I

-Azoamino benzenes[7]	Total N, % Calcd.	Found		Diazoamino N, % Calcd.	Found	
Di-	21.32	21.36	21.23	14.21	14.26	14.38
p-Methyldi-	19.90	19.90	19.84	13.25	13.26	13.35
p-Nitrodi-	23.14	23.03	23.09	11.57	11.47	11.55
p,p'-Dichlorodi-	15.80	15.77	15.90	10.53	10.60	10.51

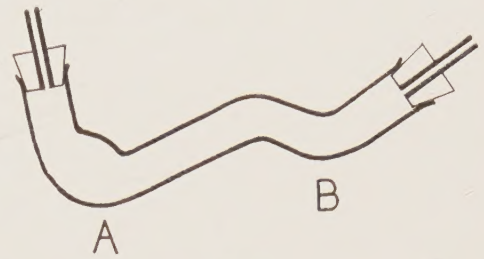

Fig. 1.

Summary

1. In a further attempt to prepare a nerve-cell staining hypnotic derivative of 5-phenyl-5-ethylbarbituric acid, three new colored derivatives of the ureide have been synthesized. These products did not exhibit the desired physiological action. 5-*m*-Hydroxyphenyl-5-ethylbarbituric acid, a white substance used as an intermediate, also was shown to be devoid of hypnotic activity.

2. A method for the microdetermination of diazoamino nitrogen has been devised and tested.

Acknowledgment

The writer wishes to express his sincere appreciation of the helpful guidance and kind cooperation of Dr. Mary M. Rising, under whose direction this research was carried on.

(5) Houben-Weyl, "Die Methoden der organischen Chemie," Second edition, Leipzig, Vol. IV, 1924, p. 668.

(6) Pregl, "Quantitative Organic Microanalysis," second ed. (Fyleman), P. Blakiston's Son, Philadelphia, 1930.

(7) The pure diazoamino compounds were kindly prepared by Mr. W. A. Erickson.

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